A CRITICAL STUDY OF THE ORGANISMS CULTIVATED FROM THE LESIONS OF HUMAN LEPROSY, WITH A CONSIDERATION OF THEIR ETIOLOGICAL SIGNIFICANCE.*†

CHARLES W. DUVAL, M.D.

AND

CREIGHTON WELLMAN, M.D

From the Laboratories of Pathology and Bacteriology and from the Laboratories of Tropical Medicine and Hygiene[Study No. 19], Medical Department, Tulane University.)

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INTRODUCTION.

In a recent communication the writers[1] announced that more than one strain of acid-fast bacilli could be cultivated by special methods from the lesion of human leprosy, and drew special attention to a non-chromogenic form which cannot be made to adapt itself to a vegetative habit and even though many generations removed from the parent stem will not grow on ordinary media, nor, indeed, on any but specially prepared nutrients containing broken-down protein.

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[1] *Jour. Am. Med. Ass.*, 1912, 58, p. 1427.

A preliminary study of the different strains of organisms encountered in the course of our investigations of leprosy from the standpoint of its bacteriology convinced us that further research on the types of organisms cultivated was necessary, and the present paper is the result of a comparative consideration of the various strains isolated from leprous patients by different workers.

It is our intention to continue our investigations on this subject until we have attempted to settle finally the various mooted points mentioned in the following paragraphs.

OCCASION AND SCOPE OF THE PRESENT PAPER.

In the course of our work we have isolated and grown from eight different cases of leprosy an acid-fast bacillus which is non-chromogenic and cannot be cultivated in our hands except in the presence of an amino-acid medium. This strain, which was described somewhat in detail by one of us (Duval[1]) in 1910, is entirely unlike the chromogenic leprosy cultures subsequently isolated by ourselves and others.

A review of the literature, together with a careful study of our cultures and preparations, has convinced us that two and possibly three apparently different organisms have been cultivated from the specific lesions of leprosy, namely: (1) a non-acid-fast diphtheroid (Kedrowski), (2) an acid-fast chromogenic bacillus (Clegg), and (3) a permanently acid-fast bacillus (Duval) which *in vitro* maintains, well within specific limits as accepted, for instance, for *B. tuberculosis*, the morphology of the Hansen bacillus of the tissues and grows under artificial conditions only in the presence of special nutrients.

Whether the three varieties described by the above authors represent the same or distinct species, some one of which is the real exciter of leprosy and the others simply extraneous or accidental commensals, is a problem which we have attempted to solve by a comparative study of the lesions induced experimentally, by the behavior of the cultures with respect to immune sera, and by other well known methods. Furthermore we have gone back over some of the leprosy cases formerly studied to determine, if possible,

[1] *Jour. Exper. Med.*, 1910, 12, p. 649.

which yield the chromogenic and which the non-chromogenic strains above mentioned, or whether from any case the two types may be cultivated.

In other words, we have attempted to answer the following queries:

1. In what percentage of the lepers observed by us and at what stage and type of the disease does the chromogenic strain (Clegg), the non-chromogenic type (Duval), and the diphtheroid type (Kedrowski) exist in the lesions, and do any or all of the acid-fast strains grow outside of the animal body under conditions as non-acid-fast diphtheroids?

2. Are streptothrical forms cultivated from lepers in this region?

3. What are the relations between the chromogen of Clegg and the non-chromogen of Duval from the leprous lesion and what are the relations of both to other known acid-fast bacteria?

4. What is the value of animal experiments and serological tests in differentiating the bacteria isolated from leprous lesions?

5. Can an etiological rôle for any of the cultures studied by us be established, to the exclusion of the others, either by means of serum reactions or by a study of histo-pathological differences in the experimental lesion?

HISTORICAL.

Following upon the discovery by Hansen[1] in 1872 of an acid-fast bacillus in the leprous lesion to which he ascribed an etiological rôle, numerous investigators reported success upon the artificial cultivation of the specific organism of leprosy.

In general it may be stated that the earlier workers, among whom may be mentioned Bordoni-Uffreduzzi,[2] Babes,[3] Ducrey,[4] Czaplewski,[5] Spronck,[6] and others, isolated and described cultures which tinctorially and morphologically differed from the Hansen bacillus of the tissues, and though they claimed to have induced experimental lesions and to have fulfilled other postulates their results have not been universally accepted.

Kedrowski[7] in 1901 described an organism which he cultivated from the leprous lesion and believed to be the specific bacillus of leprosy. This author described his culture as a non-acid-fast diphtheroid bacillus which when injected into laboratory animals became acid-fast after a sojourn of weeks in the tissues. He advanced the theory that the acid-fast rods seen in human leprous lesions represent but a stage in the developmental cycle of a single pleomorphic species.

[1] *Norsk. Mag. f. Laegevidensk.*, 1874.

[2] *Ztschr. f. Hyg.*, 1884, 3, p. 178.

[3] *Ztschr. f. Hyg.*, 1889, 5, p. 173.
Giorno italiano dello Med. ver., 1892, 27, p. 76.

[5] *Centralbl. f. Bakt.*, 1897, 23, p. 97.

[6] *Semaine méd.*, 1898, 18, p. 393.

[7] *Ztschr. f. Hyg. u. Infectionskr.*, 1901, 37, p. 52

Deycke[1] and also Rost and Williams[2] have since reported upon the successful cultivation from the leprous nodule an organism similar to that of Kedrowski together with which they also found streptothrical forms and acid-fast rods.

More recently (1912) Bayon[3] describes a non-acid-fast diphtheroid obtained from a leper which behaves in a like manner to Kedrowski's, i.e., the initial growth from the tissues is non-acid-fast and a diphtheroid until passed through rats, after which it permanently changes into a typical acid-fast bacillus. Like Rost and Williams, he also mentions streptothrical forms in his culture. He concludes that not only is his culture identical with Kedrowski's but also that it is the cause of human leprosy, basing his argument upon specific reactions obtained with human leper serum and also upon the production of characteristic lesions in laboratory animals.

Clegg[4] in 1909 announced his success in the cultivation of an acid-fast bacillus which he isolated from lesions in a large series of lepers in the Philippines. He reported that multiplication in each instance occurred in the transferred leprous tissue bits when planted with amebae and their symbionts. He subsequently obtained pure cultures of the acid-fast organisms on the ordinary laboratory media as a moist profuse pigmented growth after heating at 60° C. for 30 minutes to kill out the symbionts.

Duval[5] (1910) was the first to confirm Clegg's work, and described a method by which the acid-fast bacilli in the leprous lesion could be cultivated *in vitro* without the use of symbionts. Duval's culture differed from Clegg's in that it did not produce pigment and refused to grow except upon special nutrients.

Acid-fast cultures similar in every respect to Clegg's have since been reported by Duval[6] in Louisiana, Brinkerhoff, and Currie[7] in Honolulu, Rivas[8] in Philadelphia, Thompson[9] in Australia, Wellman[10] in California, and by other workers in Hawaii.

CRITICAL NOTE.

We wish particularly to draw attention here to the curious results obtained by various workers with leprosy organisms who believe that they have in pure culture bacteria of such protean pleomorphism that these may alternately appear as non-acid-fast diphtheroids, as both acid-fast and non-acid-fast streptothrices, and as ordinary acid-fast rods. For instance, Bayon,[11] after detailing his somewhat disheartening experience with various methods of study, writes: "I now took in hand the non-acid-fast streptothrix and the acid-resisting diphtheroid I had cultivated from a leper at this school, injecting them into rats and mice. After periods vary-

[1] *Ges. Dtschr. Naturf. u. Arzte*, 1910.

[2] *Scientific Memoirs of the Government of India*, 1911, 42, p. 1.

[3] *Tr. Soc. Trop. Med. and Hyg.*, 1912, 5, p. 158.

[4] *Philippine Jour. Sc.*, 1909, 4, p. 403.

[5] *Loc. cit.* [6] *Jour. Exper. Med.*, 1912, 15, p. 292. [7] U.S. Government Reports, 1910.

[8] Paper read before the American Association of Pathologists and Bacteriologists, Philadelphia, 1912.

[9] *Australian M. Gazette*, 1912, 31, p. 209. [10] Personal communication. [11] *Loc. cit.*

ing from three weeks to several months, they were found to have turned into acid-fast rods."

Bayon attempts to support his position by reference to the experiments of Sanfelice with *Streptothrix alba* during which it was found that this organism can break up into cultivable acid-fast rods in the animal body.

Before Bayon's work Rost,[1] Williams,[2] Kedrowski,[3] and others had cultivated organisms other than acid-fast rods from leprous lesions and in the course of our investigations such organisms have come to light. Reference to these will be made later. The work of Rost and Williams is considered by them to show that the organism of leprosy (called *Streptothrix leproides*) is "an extremely pleomorphic streptothrix which under certain circumstances may be: (1) a non-acid-fast streptothrix with interlacing filaments; (2) a non-acid-fast diphtheroid bacillus, which is in reality a streptothrix, and capable of becoming acid-fast under certain defined conditions; (3) a definite acid-fast filamentous streptothrix, and (4) an acid-fast bacillus which is the broken-down stage of a streptothrix."

Now what is the explanation of these astounding findings? In the light of our own work it appears to be simple. We have met with the diphtheroid of Kedrowski in leprous lesions and have had no difficulty in obtaining it in pure culture, which we have by us at this writing, many generations from the parent culture. It has no tendency to become acid-fast either in the most diverse culture media or in the animal body. We regard it as a distinct organism and can find no evidence that it is etiologically related to leprosy. It is closely related to the well known group of diphtheroids (*B. pseudo-diphtheriticus*, *B. xerosis*, *B. gangosae*, etc.) which can be cultivated from various sources.

The branching filamentous and interlacing non-acid-fast and acid-fast streptothrices we have never found during a careful and exhaustive bacteriological examination of 29 cases of leprosy, and we are prone to consider it a contaminator.

To any who object to such explanations we would ask this question: Why is it that cultures obtained from leprous lesions, and containing one or both of these organisms, if injected into

[1] *Loc. cit.* [2] *Indian Med. Gazette*, 1911, 46, p. 249. [3] *Loc. cit.*

animals "turn into acid-fast rods"? We also ask: Why is it again, when the organisms are "recovered" from the animal, that no medium in the wide repertoire of the bacteriologist can persuade them to assume again their supposed normal streptothrical character when outside of the tissues? The answer is again simple. By passing the culture through the animal body one gets rid of the diphtheroid and streptothrical contaminators and recovers in pure culture what before was mixed growth, namely, the acid-fast bacillus of which we think there are two distinct species, the strictly parasitic and much more delicate of these being exceedingly apt to elude all but the most careful and special technic for its recognition where it is associated with another acid-fast species.

Such an explanation is in entire accord with the experience of all bacteriologists who, as is well known, commonly employ the device of passing cultures through animals in order to separate and secure in pure growth some particular species.

The bewildering number of "stages" in the supposedly single organism of the writers above mentioned presupposes such a sweeping change in all our ideas of biological analogy that only a most cautious and critical attitude is permissible when discussing these theories.

AUTHORS' RECENT RESEARCHES.

In the course of the work we have attempted the cultivation of the Hansen bacillus from 29 cases of leprosy and have succeeded in isolating an acid-fast bacillus from 22 of these cases. The chromogenic variety (Clegg) was recovered from 14 of these cases while eight yielded a non-chromogenic acid-fast bacillus which thus far has refused to produce pigment or multiply *in vitro* on ordinary laboratory media.

For many generations the subplants both of the chromogenic and of the non-pigment-producing forms have each remained well within the morphological variations of a species and have in general maintained pretty closely the morphology of the Hansen bacillus as we know it in the human lesion.

In the 14 cases above mentioned, the acid-fast culture recovered has eventually undergone a marked change in morphology and

cultural features[1] after which it could be propagated upon the ordinary laboratory media. These chromogenic cultures correspond to those first isolated by Clegg.

In the eight cases referred to, the non-chromogenic culture, although behaving much as did the Clegg chromogenic bacillus for the first two or three months under artificial growth conditions, has refused to alter in a similar manner. This bacillus grows well on an amino-acid medium but, unlike the Clegg culture, will not multiply on ordinary laboratory foodstuff.

Since the chromogenic culture behaved much in the same manner during the first three or four months under artificial cultivation, we have looked for a similar change to occur eventually for this "slow-growing" strain. It would seem, however, that it will not do so, as the period of parasitism experienced by the cultures which subsequently became chromogenic and vegetative has long passed. The oldest culture of this slow-growing, non-chromogenic bacillus has now been under cultivation for more than two years, but has attracted special attention only in the past nine months, because it persists in refusing to alter in any respect, though more than 50 generations removed from the parent stem. We have carried out exact studies (*vide infra*) to ascertain the minimum amount of amino-acid papulum necessary to keep this strain growing and have failed to do so upon any medium known to us except that containing split products of protein digestion.

In view of the delicate character of the growth of this organism and its conformity more to our ideas of a parasitic species than the chromogen, and thinking that the chromogenic strain in certain cases might possibly be mixed with it and thus account for the production of characteristic lesions in the monkey by Duval and Couret working with the Clegg type of culture, we have replated these cultures and find that the particular culture employed actually did contain the slow-growing non-chromogenic bacillus in symbiosis with, and overgrown by, the more vigorous chromogenic form.

In view of the significance attached by us at present to this non-pigment-producing strain which was first isolated from leprous

[1] *Arch. Inter. Med.*, 1911, 7, p. 230.

lesions and described by one of us (Duval) in 1910, we have thought best briefly to describe it again formally and to place cultures in the hands of other competent workers so that its exact status and importance may be settled.

DESCRIPTION OF ORGANISM.

The following brief résumé of the bacillus now being specially studied by us is here presented for comparison and reference. (For detailed description see Duval, *Jour. Exper. Med.*, 1910, 7, p. 649.)

Source of culture: subcutaneous leprosy nodule.

Date of isolation: November 10, 1909.

Morphology.—Vegetative cells on amino-acid medium at 37° C. These vary from short plump to long slender rods. The chromatin is irregularly placed, often resulting in a beaded and bipolar effect. Clear non-staining areas are in consequence also irregularly disposed.

Limits of size: $2-5\,\mu \times 0.2-0.4\,\mu$.

Endospores absent.

Flagella absent.

Capsule absent.

Involution forms: These may be seen in symbiotic cultures, in old pure cultures, and in cultures where the placental extract is diluted with other media (*vide infra* under "Special Methods of Cultivation").

Staining reactions: Takes the ordinary basic stains, is gram positive and acid-fast.

Cultural features.—(1) On amino-acid agar stroke culture is of scanty growth, moist, filiform, flat, glistening, smooth, and somewhat opaque.

Chromogenesis: None.

(2) In placental extract: surface growth, none under ordinary circumstances (floats are at the present writing being tried).

Clouding slight.

Sediment: moderately abundant.

Pathogenicity.—(See section on animal experiments.)

Brief characterization.—A gram positive and acid-fast, non-motile, moderately slender bacillus growing only on special media and generally presenting in culture the morphology of *B. diphtheriae*.

As to the inability of this bacillus to grow on ordinary media it may be said that we have undertaken exact experiments to determine the minimum amount of amino-acid foodstuff (placental extract) necessary to grow it upon solid media. Dilutions were made as follows:

Placental extract	1	part;	3 per cent sterile agar	1	part.
"	"	1	"	nutrient agar	2	parts.
"	"	1	"	"	"	3	"
"	"	1	"	"	"	4	"
"	"	1	"	"	"	5	"
"	"	1	"	"	"	6	"
"	"	1	"	"	"	7	"
"	"	1	"	"	"	8	"
"	"	1	"	"	"	9	"
"	"	1	"	"	"	10	"

Transplants from the original medium (placental extract 1 part, sterile agar 1 part) to the other dilutions showed that the organism will grow upon 1 part in 7 of placental extract with nutrient agar but will not multiply upon 1 part in 9 or 10.

SPECIAL METHODS OF CULTIVATION.

The initial multiplication of both the acid-fast strains referred to in this paper is accomplished with comparative ease provided that the bits of leprous tissues transferred are treated in such a way that the protein moiety is split into its dissociate products.

This action upon the protein of the removed leprous lesions may be accomplished in the following ways: (1) by seeding the tissue transplants with some one of the putrefactive bacteria or with any species capable of hydrolizing the tissues; (2) by saturating the removed tissue bits with 1 per cent trypsinized albumen solution; (3) by transferring the leprous material directly to a medium containing the products of protein digestion (placenta-extract agar).

With any of these methods the acid-fast bacilli in the removed lesions will multiply and continue to do so as long as these products are present, which of course is permanently in the case of the medium last mentioned.

In several instances we have failed to obtain any growth of acid-fast bacilli from leper patients. In one such instance the diphtheroid non-acid-fast organism (Kedrowski) was cultivated from the lesions. In these cases it may be mentioned that no acid-fast bacilli were demonstrable in stained smear preparations from the removed and macerated bits of tissue, although clinically the patients were regarded as typical lepers.

While perhaps the hydrolizing method offers the most certain means of obtaining the initial multiplication of the acid-fast bacilli, the placenta-extract agar is to be preferred since, especially in the case of the non-chromogenic bacillus, it does not necessitate replating and minimizes the chance of contamination. In fact we have found that even where the tissue is first hydrolized the addition of an amino-acid solution, such as placental juice, is distinctly advantageous, especially if glycerin is added, as the latter holds in check the growth of the hydrolizer.

When a hydrolizer alone is used continued multiplication is attended with the greatest difficulty as soon as the original split

products are exhausted, and even though placental extract or other amino-acids are substituted at this stage the growth activity is slower than in the initial period, taking from three to four weeks to attain its maximum.

EXPERIMENTAL LESIONS WITH DIFFERENT LEPROSY CULTURES.

In the course of the present study we have employed in our experiments 42 rabbits, 48 guinea-pigs, 35 white mice, 14 white rats, and two monkeys.

To determine if possible whether gross or microscopic differences exist for the experimental lesion induced by the two varieties of acid-fast bacilli which have been isolated and cultivated from the human leprous lesion, or whether the experimental lesions induced with either strain are similar to the human lesion or to those produced by the well known saprophytic acid-fast species, rabbits, guinea-pigs, and rats were injected with graduated doses of the following cultures, namely, five chromogenic leprosy cultures (Clegg, Duval, Brinkerhoff, Currie, and Bayon respectively), the non-chromogenic slow-growing lepra culture, and two representative saprophytic species (Mueller's grass bacillus and the bacillus of timothy hay).

The rabbits were inoculated intravenously, using 5 c.c. of a heavy homogeneous emulsion of the various cultures named. The animals received in all four injections at weekly intervals and were killed three months after the first inoculation. Similar experiments were carried out upon a series of guinea-pigs and white rats. In the series of guinea-pigs two injections were administered subcutaneously at intervals of 14 days, using 2 c.c. of a heavy homogeneous suspension for the first, and 4 c.c. for the second injection. The rats received intraperitoneally the same quantity of culture and number of injections.

The rabbits showed at autopsy microscopic lesions in the lungs, liver, spleen, and kidneys, irrespective of the culture employed, though macroscopic lesions were not demonstrable in all of the organs mentioned. The most marked gross lesions occurred in the rabbit injected with the bacillus of timothy hay and the chromogenic lepra cultures (Currie and Bayon). The most pronounced

and extensive lesions occurred in the rabbit which had received injections of the bacillus of timothy hay. In general the lung lesions resemble on naked eye appearance the miliary tubercle, while lesions in the liver are larger and of a somewhat different character. Here they resemble small healed gummata, with centers composed of a dry granular salmon-colored material surrounded by a dense tough zone of fibrous tissue.

The rabbits which received injections of the non-chromogenic culture of *B. leprae* showed no macroscopic evidence of lesions; however, the sections from the liver and spleen reveal definite areas in which the nuclei of the parenchymatous cell are broken and fragmented and the whole area crowded with acid-fast bacilli. For the most part the bacilli are scattered, with here and there dense collections contained within large cells and resembling the so-called leprosy globi. Although similar in some respects to the human leprous lesion in the internal organs and to those induced in the Japanese dancing mouse (Duval), they lack the histological picture (proliferative type of lesion) which characterizes the human leprous nodule.

The guinea-pigs were killed four months after the date of the first inoculation and in no case were there detected gross lesions except at the site of inoculation. In the animals which received the non-chromogenic leprosy culture there existed at the site of the second inoculation a small fibrous nodule, approximately the size of a split pea, which on microscopic examination contained a moderate number of acid-fast bacilli for the most part extracellular. The cell picture was not entirely characteristic of the human subcutaneous leprous nodule; however, it did resemble it in that there was no caseation and it contained many cells of the epithelioid type.

The guinea-pig which received Clegg's chromogenic lepra culture showed at the site of the second inoculation a fluctuating nodule the size of a marble. This area when opened discharged a thick, creamy yellow necrotic material and was found to contain enormous numbers of acid-fast bacilli in pure culture. The animal showed no microscopic lesions in the internal organs.

The series of white rats revealed no macroscopic lesions at

autopsy. However, small microscopic foci of lymphoid and plasma cells associated with scattered and clustered acid-fast bacilli were demonstrable on microscopic examination in sections from the omentum, spleen, and liver in one rat which had received the non-chromogenic lepra culture.

In general it may be stated that macroscopically the lesions produced in rabbits by intravenous injection do not differ greatly for any of the species of acid-fast cultures employed, unless it be that the chromogenic culture produces lesions which appear earlier and are more localized. With this culture the lung usually shows the most extensive change in the form of grayish-white discrete foci indistinguishable from miliary tubercles except possibly for the absence of necrosis. Microscopically the cell picture or relation of the bacilli with respect to the cells is not sufficiently distinctive of any culture employed to warrant more than a tentative differentiation. Although relative histological differences are detected for the experimental lesion, the difference is largely one of degree.

Suffice it to say that from our studies so far upon the experimental animals there is no absolute differentiation of the lesion induced by any given strain or species of acid-fast organism, excluding, of course, the tubercle family. A comparative study of the experimental lesions produced by the various acid-fast species is at present in progress by Dr. Couret in collaboration with one of us (Duval). Lesions are as readily induced experimentally with some of the well known saprophytic species as they are induced with either the infested leprous tissue or with lepra culture.

The five rabbit protocols here given are typical of the entire series and represent the results obtained by intravenous injections of the various cultures employed. An examination of these records will show the character and distribution of the induced lesions. All the animals of this series received at the same time and under the same conditions four doses of the respective cultures mentioned in the protocols.

Experiment 20. Rabbit A: Black female rabbit which had received four intravenous injections (ear vein) of an emulsion of *B. leprae* (Honolulu) on February 28, March 31, April 11, and April 22, respectively. The dose at each injection was approximately four billion bacilli. On April 14, the rabbit was bled from the heart and 4 c.c.

of blood removed. The animal steadily lost weight and died three months after the date of the first inoculation.

Gross examination.—The mesentery contains small firm nodules with small yellowish centers, ranging from 1 to 2 mm. in diameter. Several of the retroperitoneal lymph nodes are enlarged and on section show numerous yellow-colored necrotic areas. Smears from the necrotic material show innumerable scattered and dense colony masses of acid-fast bacilli. The lungs show extensive areas of hemorrhage and small well defined areas of consolidation. The peribronchial lymph nodes are enlarged but on section show nothing remarkable. On the right and left ventricles of the heart just beneath the epicardium are several small discrete yellowish areas similar to those in the mesentery. The liver contains many partially fibrosed gummatoid areas, the largest measuring 6 mm. in diameter. These areas in the liver are sharply defined and consist of an outer pearly gray translucent zone 1 mm. in thickness, and a central necrotic area which is of an orange-yellow color. Smears prepared from these necrotic centers show innumerable acid-fast bacilli. The other organs appear normal.

Microscopic examination.—The smaller lesions are made up of lymphoid and plasma cells and contain enormous numbers of acid-fast bacilli. In the more advanced lesions the central portion is composed of broken and fragmented nuclei, showing, however, no inflammatory reaction, while the lesion at the periphery is composed of epithelioid cells and fibroblastic elements forming a fairly dense encapsulating zone.

Rabbit B: Gray female rabbit which had received intravenously into the ear veins four injections of an emulsion of *B. leprae* (non-chrom.). The dose in each instance was approximately four billion bacilli which was injected on the following dates: February 28, March 31, April 11, and April 22. On May 14 the rabbit was bled and 4 c.c. of blood removed. The animal at this time was well nourished, vigorous, and showed no evidence of disease. Two months and a half after the first injection the rabbit was killed and at autopsy showed no demonstrable gross lesions except in the liver, where an occasional grayish-white poorly defined lesion was detected. Scrapings from these foci showed numerous acid-fast bacilli.

Microscopic examination.—Sections from the liver, spleen, and kidney show small areas of broken and fragmented cells and polymorphonuclear leukocytes and enormous collections of acid-fast bacilli. In no instance is there any fibrous tissue proliferation or presence of the epithelioid type of cell.

Rabbit C: Gray male rabbit which had been given four intravenous injections of an emulsion of *B. leprae* (Bayon) on the same dates as for the other animals of the series. The dose was approximately four billion bacilli. On April 14 the rabbit was bled from the heart and 4 c.c. of blood removed. The rabbit was killed three and one-half months after the first inoculation and at the time was well nourished and showed no evidence of disease.

Gross examination.—No gross lesions are detected except in the liver, where there is an occasional grayish-white area somewhat necrotic in the center. Smear preparations from this material shows enormous numbers of acid-fast bacilli.

Microscopic examination.—The sections from the various organs are negative except for the liver. Here the lesion is composed of lymphoid and epithelioid cells in and among which are numbers of acid-fast bacilli.

Rabbit D: A yellow and white female rabbit which had received intravenously on February 28, March 31, April 11, and April 22 four billion bacilli of the culture

bacillus of timothy hay. On April 14 the rabbit was bled from the heart and 4 c.c. of blood removed. The animal was killed three and one-half months after the first inoculation.

Gross examination.—At autopsy the lungs, liver, spleen, kidney, mesentery lymph nodes, mesentery, and omentum contain innumerable raised grayish-white necrotic foci ranging from pin-point to 2 mm. in diameter. The resemblance of the lesions in the lungs to miliary tubercles is striking. Acid-fast bacilli exist in these lesions in small numbers.

Microscopic examination.—The young lesions are composed of necrotic tissue, moderate number of polynuclears and giant cells, while older nodules show extensive necrosis, epithelioid formation, and infiltration with acute inflammatory elements.

Rabbit E: Black male rabbit which had received on four occasions an intravenous injection of *B. leprae* (Clegg). The dose was approximately four billion bacilli. The animal was killed three and one-half months after the first injection.

Gross examination.—In the ears where the injections had been made there is a marked phlebitis and thickening of the surrounding tissues. Smears from this location show enormous numbers of acid-fast bacilli which are for the most part in dense clusters or globi. On the left ventricle of the heart is a small hard white patch about the size of the head of a pin which also contain numerous acid-fast bacilli. This patch is well circumscribed and shows no evidence of necrosis. The other organs appear normal.

Microscopic examination.—The lesions are distinctly proliferative in character and occur in the liver, spleen, and kidney. The histological picture is indistinguishable from the human leprous nodule. In general they consist of a dense mosaic of epithelioid cells, interspersed with lepra cells containing globi.

Rabbit F: A white and black rabbit was injected intravenously on four separate occasions (February 28, March 31, April 11, and April 22) with approximately four billion bacilli of *B. leprae* (Currie). On April 14 the rabbit was bled from the heart and 4 c.c. of blood removed. The animal was well nourished and showed no evidence of disease. At autopsy gross lesions in the form of discrete areas 1 to 2 mm. in diameter are detected in the liver, spleen, and kidney. The other organs are apparently negative.

Microscopic examination.—The lesions do not differ in character from those described for Rabbit E.

SEROLOGICAL TESTS WITH DIFFERENT STRAINS OF ORGANISMS.

With the view of determining a possible relationship between the acid-fast chromogen, the non-chromogenic acid-fast, and the saprophytic chromogenic species, Dr. William H. Harris and John A. Lanford of the Department of Pathology have carried out an exhaustive serological study, which will shortly be published. A series of rabbits were immunized with the respective cultures and a comparative study carried out upon the immune sera for the detection of specific antibodies. Realizing the difficulty of immunizing against the acid-fast group the animals were subjected

to a long period of treatment, administering large doses intravenously at weekly intervals over a period of three months.

In addition the blood from a series of 20 cases of leprosy was also tested for specific antibodies to determine if possible a specificity for any given culture isolated from the same case or from other cases of leprosy. In performing these serum tests the agglutination reaction and the complement-binding tests, using a culture antigen, were employed.

Their results show that the serological tests with the blood of lepers has not established an etiological rôle for either type of acid-fast organism recovered from the leprous lesion. The agglutination reaction with the lepers' blood rarely gives a positive reaction in dilution of 1/50 with the separated Hansen bacilli obtained from the human nodule, while in the majority of cases a reaction is not obtained above a dilution 1/10. On the other hand, many of the tubercle family and the acid-fast saprophytes react equally as well and not infrequently in higher dilutions (see Table 1). The Wassermann reaction with culture antigen utterly fails to show anything specific for the two varieties of culture in so far as the human serum is concerned. However, the serum reaction of animals immunized against the various acid-fast species has served to separate into three distinct groups, namely, the chromogenic culture of leprosy (Group I), the non-chromogenic culture of leprosy (Group II), and the chromogenic saprophytic acid-fast species (Group III).

The reaction with specific immune serum establishes the fact that there is a difference between the non-chromogenic and the chromogen leprosy cultures. Furthermore the serum reaction indicates no relation between these two strains and any saprophytic species.

The following tables are from the series of leprosy cases mentioned above and are typical of the entire series, including examples of reactions both in high and in low dilutions.

The tests consisted (1) of the reactions of the patients' own blood with 15 strains of acid-fast bacilli; (2) of the agglutination reactions and complement fixation tests with specific immune sera.

In order to avoid repetition and the use of unnecessary space only three tables will be given. They represent the results of tests

made with the sera obtained from cases of human leprosy and with specific immune sera of rabbits.

TABLE 1.

AGGLUTINATION REACTIONS WITH SERUM OF LEPERS.

LEPROSY CASE	CULTURE (SUSPENSION)	SERUM DILUTION	RESULT		REMARKS
			1 Hour	Final	
II. Brunner. Trophic type. Duration 10 years	1. Non-chrom	1:10	+	+	Positive reaction with chromogenic leprosy cultures generally in higher dilution than with other species. This case had received several injections of the protein extract of Culture 3 (chromogen, Duval).
	2. Chrom. (Clegg)	1:160	+	+	
	3. Chrom. (Duval)	1:160	+	+	
	4. Chrom. (Currie)	1:160	+	+	
	5. Chrom. (Brinkerhoff)	1:10	+	+	
	6. Chrom. ("Hawaii")	1:80	+	+	
	7. Chrom. (Bayon I)	1:80	+	+	
	8. Tubercle (human)	1:40	+	+	
	9. Tubercle (bovine)	1:10	+	+	
	10. Tubercle (avian)	1:10	+	+	
	11. Timothy hay (dry)	1:10	+	+	
	12. Mueller's No. 1	1:10	+	+	
	13. Mueller's No. 2	1:10	+	+	
	14. Korn	1:10	+	+	
	15. Karlinski	1:10	+	+	
VIII. Fritz. Nodular type. Duration 5 years	1. Non-chrom	1:80	+	+	All acid-fast cultures used agglutinate with this serum except the "dry" growers (B. timothy hay and Karlinski).
	2. Chrom. (Clegg)	1:80	+	+	
	3. Chrom. (Duval)	1:80	+	+	
	4. Chrom. (Currie)	1:10	+	+	
	5. Chrom. (Brinkerhoff)	1:40	+	+	
	6. Chrom. ("Hawaii")	1:40	+	+	
	7. Chrom. (Bayon)	1:20	+	+	
	8. Tubercle (human)	1:20	+	+	
	9. Tubercle (bovine)	1:80	+	+	
	10. Tubercle (avian)	1:40	+	+	
	11. Timothy hay (dry)	1:10	−	−	
	12. Mueller's No. 1	1:70	+	+	
	13. Mueller's No. 2	1:40	+	+	
	14. Korn	1:20	+	+	
	15. Karlinski	1:10	−	−	
XIV. Moore. Maculo-anesthetic type. Duration 4 years	1. Non-chrom	1:40	+	+	Good agglutinations throughout the series with this serum.
	2. Chrom. (Clegg)	1:40	+	+	
	3. Chrom. (Duval)	1:80	+	+	
	4. Chrom. (Currie)	1:80	+	+	
	5. Chrom. (Brinkerhoff)	1:80	+	+	
	6. Chrom. ("Hawaii")	1:40	+	+	
	7. Chrom. (Bayon)	1:40	+	+	
	8. Tubercle (human)	1:40	+	+	
	9. Tubercle (bovine)	1:40	+	+	
	10. Tubercle (avian)	1:40	+	+	
	11. Timothy hay (dry)	1:40	+	+	
	12. Mueller's No. 1	1:80	+	+	
	13. Mueller's No. 2	1:20	+	+	
	14. Korn	1:70	+	+	
	15. Karlinski	1:50	+	+	
XII. Chevalier. Mixed type. Duration 8 years	1. Non-chrom	1:10	−	+	Low agglutinations throughout except with 8 and 13. This case received subcutaneously the protein extract of 3 (chrom. Duval) over a period of one year.
	2. Chrom. (Clegg)	1:40	+	+	
	3. Chrom. (Duval)	1:40	+	+	
	4. Chrom. (Currie)	1:10	+	+	
	5. Chrom. (Brinkerhoff)	1:40	+	+	
	6. Chrom. ("Hawaii")	1:10	+	+	
	7. Chrom. (Bayon)	1:10	+	+	
	8. Tubercle (human)	1:150	+	+	
	9. Tubercle (bovine)	1:40	+	+	
	10. Tubercle (avian)	1:10	+	+	
	11. Timothy hay (dry)	1:10	+	+	
	12. Mueller's No. 1	1:80	+	+	
	13. Mueller's No. 2	1:20	+	+	
	14. Korn	1:70	+	+	
	15. Karlinski	1:50	+	+	

TABLE 1.—*Continued.*

Leprosy Case	Culture (Suspension)	Serum Dilution	Result		Remarks
			1 Hour	Final	
VI. Amelia. Tubercular type. Duration 3 years	1. Non-chrom	1:80	+	+	Avian tubercle and Mueller's grass bacillus react equally as well with this serum as the non-chrom. leprosy culture.
	2. Chrom. (Clegg)	1:60	+	+	
	3. Chrom. (Duval)	1:40	+	+	
	4. Chrom. (Currie)	1:40	+	+	
	5. Chrom. (Brinkerhoff)	1:40	+	+	
	6. Chrom. ("Hawaii")	1:10	+	+	
	7. Chrom. (Bayon)	1:40	+	+	
	8. Tubercle (human)	1:40	+	+	
	9. Tubercle (bovine)	1:10	−	−	
	10. Tubercle (avian)	1:80	+	+	
	11. Timothy hay (dry)	1:10	−	−	
	12. Mueller's No. 1	1:80	+	+	
	13. Mueller's No. 2	1:10	+	+	
	14. Korn	1:40	+	+	
	15. Karlinski	1:10	−	−	
VII. Pablo. Maculoanesthetic type. Duration 3 years	1. Non-chrom	1:10	+	+	All cultures react in low dilutions except with Mueller's grass bacillus No. 1 which gives the highest reaction.
	2. Chrom. (Clegg)	1:10	+	+	
	3. Chrom. (Duval)	1:20	+	−	
	4. Chrom. (Currie)	1:10	+	+	
	5. Chrom. (Brinkerhoff)	1:40	+	+	
	6. Chrom. ("Hawaii")	1:10	−	+	
	7. Chrom. (Bayon)	1:10	+	+	
	8. Tubercle (human)	1:10	+	+	
	9. Tubercle (bovine)	1:10	+	+	
	10 Tubercle (avian)	1:10	+	+	
	11. Timothy hay (dry)	1:10	−	+	
	12. Mueller's No. 1	1:60	+	+	
	13. Mueller's No. 2	1:10	+	+	
	14. Korn	1:10	+	+	
	15. Karlinski	1:10	+	+	

TABLE 2.

AGGLUTINATION REACTION WITH ANTI-LEPROSY SERA.

Immune Serum	Culture Suspension	Dilution of Sera	Results		Remarks
			1 Hour	Final	
3. Chrom. (Duval)	1. Non-chrom	1:10	−	−	
	2. Chrom. (Clegg)	1:10	+	+	
	3. Chrom. (Duval)	1:160	+	+	
	4. Chrom. (Currie)	1:10	+	+	
	7. Chrom. (Bayon)	1:10	−	+	
	11. Timothy hay (dry)	1:10	−	−	
1. Non-Chrom	1. Non-chrom	1:150	+	+	
	2. Chrom. (Clegg)	1:10	+	+	
	3. Chrom. (Duval)	1:80	+	+	
	4. Chrom. (Currie)	1:10	+	+	
	7. Chrom. (Bayon)	1:10	+	+	
	11. Timothy hay (dry)	1:10	+	+	
2. Chrom. (Clegg)	1. Non-chrom	1:10	−	−	
	2. Chrom. (Clegg)	1:50	+	+	
	3. Chrom. (Duval)	1:10	+	+	
	4. Chrom. (Currie)	1:10	+	+	
	7. Chrom. (Bayon)	1:10	+	+	
	11. Timothy hay (dry)	1:10	−	−	
4. Chrom. (Currie)	1. Non-chrom	1:10	−	+	
	2. Chrom. (Clegg)	1:40	+	+	
	3. Chrom. (Duval)	1:20	+	+	
	4. Chrom. (Currie)	1:320	+	+	
	7. Chrom. (Bayon)	1:10	+	+	
	11. Timothy hay (dry)	1:10	−	+	

TABLE 2.—*Continued.*

Immune Serum	Culture Suspension	Dilution of Sera	Results		Remarks
			1 Hour	Final	
5. Chrom. (Bayon)	1. Non-chrom.	1:10	+	+	
	2. Chrom. (Clegg)	1:20	+	+	
	3. Chrom. (Duval)	1:10	+	−	
	4. Chrom. (Currie)	1:10	+	−	
	7. Chrom. (Bayon)	1:190	+	+	
	11. Timothy hay (dry)	1:10	+	+	
6. Timothy hay (dry)	1. Non-chrom.	1:10	+	+	
	2. Chrom. (Clegg)	1:10	+	+	
	3. Chrom. (Duval)	1:10	+	+	
	4. Chrom. (Currie)	1:10	−	+	
	7. Chrom. (Bayon)	1:80	+	+	
	11. Timothy hay (dry)	1:320	+	+	

TABLE 3.

Complement Fixation Tests.

Rabbit Immune Serum	Culture Antigen	Results		Remarks
		1 Hour	24 Hours	
1. Non-chromogen	1. Non-chrom	++	++	When larger amount of antigen was employed all are −+
	2. Chrom. (Clegg)	−−	−−	
	3. Chrom. (Duval)	−−	−−	
	4. Chrom. (Currie)	−−	−−	
	7. Chrom. (Bayon)	−−	−−	
	11. Timothy hay (dry)	−−	−−	
2. Chromogen (Clegg)	1. Non-chrom	−−	−−	
	2. Chrom. (Clegg)	−+	−+	
	3. Chrom. (Duval)	−+	−+	
	4. Chrom. (Currie)	−+	−+	
	7. Chrom. (Bayon)	−+	−+	
	11. Timothy hay (dry)	−−	−−	
3. Chromogen (Duval)	1. Non-chrom	++	++	When larger amount of antigen was employed all are ++
	2. Chrom. (Clegg)	++	+−+	
	3. Chrom. (Duval)	++	+−+	
	4. Chrom. (Currie)	++	+−+	
	7. Chrom. (Bayon)	++	++	
	11. Timothy hay (dry)	−−	−−	
4. Chromogen (Currie)	1. Non-chrom	−−	−−	
	2. Chrom. (Clegg)	−−+	−−+	
	3. Chrom. (Duval)	−−+	−−+	
	4. Chrom. (Currie)	−−+	−−+	
	7. Chrom. (Bayon)	−−+	−−+	
	11. Timothy hay (dry)	−−	−−	
7. Chromogen (Bayon)	1. Non-chrom	−++	−+	
	2. Chrom. (Clegg)	−−+	−−+	
	3. Chrom. (Duval)	−−+	−−+	
	4. Chrom. (Currie)	−−+	−−+	
	7. Chrom. (Bayon)	++	++	
	11. Timothy hay (dry)	−−	−−	
11. Timothy hay (dry)	1. Non-chrom	−−	−−	When larger amount of antigen was employed all are −−+
	2. Chrom. (Clegg)	−−	−−	
	3. Chrom. (Duval)	−−	−−	
	4. Chrom. (Currie)	−−	−−	
	7. Chrom. (Bayon)	−−	−−	
	11. Timothy hay (dry)	++	++	

These rabbits received five injections of viable cultures, approximately four billion, at each inoculation and at weekly intervals over a period of four months.

++ equals very positive; −+ equals weakly positive; −− equals negative.

GENERAL DISCUSSION.

The following is the origin and number of the cultures studied by us during the course of the present investigation: (1) Clegg (four cultures), (2) Brinkerhoff (one culture), (3) Currie (two cultures), (4) Bayon (two cultures), (5) "Hawaii" (five cultures), (6) Duval (fourteen cultures), (7) Rost and Williams (one culture).

From our work with these the following considerations occur to us.

There can be little doubt that Clegg with amebae and their symbionts obtained multiplication *in vitro* of both strains of the acid-fast bacilli herein referred to, for, as already pointed out, the non-chromogenic strain will multiply in the test-tube provided the tissue bits are hydrolized. Clegg undoubtedly accomplished this through the symbionts which were associated with the amebae. His culture sent to us, which he obtained pure by heating, grew readily upon ordinary laboratory media, and contained only the chromogenic bacillus. We believe the non-chromogenic parasitic variety, if originally present, ceased to multiply after the mixed culture was transferred to ordinary media.

The occurrence of the chromogenic bacillus of Clegg in leprous lesions, especially where it occurs in the internal organs, is difficult to account for if we are to accept that it is a simple saprophyte. On the other hand, how are we to explain the occurrence of what is apparently another distinct organism in the lesions of leprosy? Are we dealing with two etiological factors, or is one the causal agent and the other an associated commensal, or do they both represent stages of the same species?

With this question in mind we have gone back over some of the cases previously examined by us (1911) to determine if possible what percentage yields the chromogenic culture and what proportion yields the non-chromogenic type or whether in any case the two are encountered. For this purpose we selected five cases of leprosy from which the chromogenic bacillus had previously been isolated. The nodules were removed with every care to avoid against extraneous contamination. Each nodule was divided into two portions, one part being hydrolized with *B. subtilis* while the other was treated with placental juice. These cultures were incubated

at 37° C. over a period of two months and examined at frequent intervals to note the character and behavior of the growth. In no case and at no time were we able to detect other than acid-fast bacilli (except of course in the tubes where *subtilis* had been added), and these acid-fast bacilli multiplied steadily and retained their acid-fastness throughout the entire period of growth. The acid-fast cultures obtained by means of the hydrolizer were subsequently obtained pure by plating on placental-extract agar. Cultures of acid-fast bacilli were recovered from all five cases. Two of these have taken on chromogenic properties and become culturally like the original isolation. The others show no tendency to alter in this respect, but conform to the description of the bacillus first described by Duval. From three of the earlier cases (1910) we have also recovered recently by plating on placental-extract agar an acid-fast culture which for months has grown slowly, is non-chromogenic, and refuses to multiply except on special media. In four other cases from which one of us (Duval) in 1910 isolated the chromogenic culture we have recently attempted a second isolation, but the cultures have all turned out to be chromogens.

It is, we repeat, hard to explain the occurrence in leprous lesions of this chromogenic acid-fast variety which in our experience with cases here is encountered more frequently than the non-chromogenic variety. This may possibly be explained by our present imperfect methods of cultivation. Curiously enough the chromogenic type, if we are to regard it as an extraneous organism, is always the same variety, i.e., a moist, rapidly growing bacillus when once it becomes accustomed to an artificial environment. We have compared the original cultures of Clegg with those isolated independently by workers in other parts of the world and find them, except for inconstant minor differences, identical. That this chromogenic species exists in the lesions of certain types of leprosy there can be no doubt, and that, too, in the lesions where the overlying skin is apparently intact as well as from the internal organs at autopsy, e.g., from the spleen.

It is interesting to note that the Clegg strain undergoes at times the most marked change in tinctorial and morphological features. It is relatively easy to obtain from this species a non-acid-fast

diphtheroid, an acid-fast beaded bacillus, and a diplococcoid acid-fast type. These cultures when grown upon an alkalin medium in the presence of symbionts are distinctly acid-fast, while the individual rods are indistinguishable from tubercle bacilli. On an acid medium in symbiosis with other bacteria they occur as non-acid-fast pleomorphic forms, many of which are distinctly diphtheroid in appearance. In pure culture on such media the individual bacilli are small acid-fast ovoid or coccoid rods occurring singly or in pairs. This wide variation in morphology and difference in staining properties might possibly account for the non-acid-fast diphtheroid "stages" described by European authors.

In the entire series of cases from which we have attempted the cultivation of the Hansen bacillus we have noted in but one case a non-acid-fast diphtheroid. This culture in our hands has failed to change into an acid-fast by passing it through rats or handling in any other manner. In other words, the non-acid-fast diphtheroid stage for the Hansen bacillus which has been described by Babes, Bordoni-Uffreduzzi, Kedrowski, and others has not occurred in our experience except in so far as the transitory changes in the Clegg culture above mentioned may be thus interpreted.

So far as the non-chromogenic, slow-growing bacillus is concerned, we believe careful attention is merited by it. In cultures it is always acid-fast and usually occurs as long and short beaded rods, although in a lesser degree the morphological change described above for the Clegg culture may be applied to this culture also. If it was not for the fact that this bacillus is always acid-fast it would be impossible to distinguish it morphologically from *B. diphtheriae*.

The presence of the chromogenic Clegg bacillus in the tissues of human lepers, together with the fact that disseminated lesions are induced experimentally which are histologically like those in human leprosy, led one of us (Duval) in a former publication to conclude that the chromogenic strain played an etiological rôle in the disease. In going back over some of the preparations from the animals employed we find them to contain the long beaded acid-fast rods in dense masses and also many more scattered bacilli which are shorter and less beaded (Clegg bacillus). These findings

were described in the paper referred to as indicating a transformation of the long slender beaded rods to diplococcoid forms, but by replating on placental media the culture employed we find it to be mixed, containing both strains, so that it is impossible to say without further work just what part the two types played in the production of lesions in the monkey. This view has been greatly strengthened by our recent results obtained by replating upon placental agar cultures from the original tryptophane fish agar formerly described by one of us (Duval). We find that in these the two varieties exist and it is therefore not improbable that the subplants of the earlier isolations upon amino-acid media would also yield both cultures.

We have not received the information we hoped for from animal experimentation. This is not surprising in view of the difficulty of inoculation experiments upon laboratory animals. The most that can be broadly stated is that, excepting *B. tuberculosis*, the lesions induced by acid-fast bacilli are not sharply differentiated. The general character of the lesions following injection of animals with the various acid-fast organisms may be briefly characterized as follows: (1) Leprosy cultures produce lesions proliferative in type without necrosis, and consist of an epithelioid cell matrix which is strongly suggestive of the human leprosy lesion. The contained bacilli are for the most part scattered and extracellular, though dense masses of organisms (globi) within large multinucleated cells occur; (2) saprophytic cultures (timothy hay, etc.) produce lesions which differ from the foregoing principally by the presence of sub-acute inflammation and extensive necrosis; (3) the experimental lesions of tuberculosis are too well known to call for remark. Even with avian cultures the lesion is distinctive enough not to confuse it with leprosy. The refractory character of laboratory animals to leprosy cultures must be borne in mind when attempting to interpret the results from experiments.

From the serological tests we have only succeeded in showing that the sera of highly immunized animals will as a rule react better to the immunizing strain than to other allied or to foreign acid-fast strains, but the reactions employing as an index either the agglutinins or the deviation of complement cannot be said to be

specific. The results in this regard are directly contradictory to those of Bayon,[1] who believed that by freezing and thawing the bacilli he could produce a satisfactory antigen which would enable him to differentiate his leprosy culture from other groups by means of the complement deviation test.

In this connection, however, attention should be drawn to the more specific character of the reaction obtained in lepers by Clegg,[2] Duval and Gurd,[3] and others who use the chromogenic leprosy cultures. A cutaneous reaction similar to that secured with tuberculin in tuberculous patients was obtained by these authors by means of injections with killed bacilli (chromogen Clegg) or the "leprosin" (protein extract) of the same. A marked constitutional reaction consisting of a rise in temperature (104° F.) and a distinct leukocytosis (15,000–24,000) was induced by the injections, together with lepra abscess formation at the site of inoculation. Acid-fast saprophytes (timothy hay) failed to give the reaction. It is possible that here we have a better means of separating the strain of leprosy cultures and that further work along these lines will yield more definite results.

SUMMARY.

From the leprous lesion two varieties of acid-fast bacilli may be cultivated, one a chromogenic pleomorphic organism which grows readily upon the ordinary laboratory media after it has become accustomed to a saprophytic existence; the other, a moist-growing non-chromogenic bacillus resembling tinctorially the tubercle bacillus, and morphologically the diphtheria bacillus, and multiplying only upon special media. The chromogenic strain, although hard to cultivate at first, subsequently grows profusely and rapidly upon a great variety of foodstuff, while the non-chromogenic strain is always difficult to cultivate and multiplies very slowly even in generations far removed from the parent stem.

The chromogenic culture may show a wide variation in morphology and its ability to retain the stain when subjected to decolorizing agents. At times and under certain conditions the individual rods are diphtheroid and non-acid-fast. The non-chromogenic culture

[1] *Loc. cit.* [2] *Phil. Med. Bull.*, 1910. [3] *Loc. cit.*

is always acid-fast and can be sharply differentiated from the chromogenic culture by its growth features.

CONCLUSIONS.

1. From a bacteriological study of 29 cases of leprosy we have isolated an acid-fast bacillus from 22 cases.

2. A chromogenic strain similar in all essentials to that described by Clegg was recovered from 14 cases.

3. Eight cases yielded an organism which is markedly different in its character from Clegg's bacillus and which will grow only on specially prepared media and refuses to become chromogenic.

4. In one case we have isolated a non-acid-fast diphtheroid bacillus corresponding to the organism described by Kedrowski.

5. We are unable to confirm the work of Rost, Williams, Bayon, and others who consider that *B. leprae* is a bacterium of such pleomorphism that it can be recognized as a diphtheroid, a streptothrix, and an acid-fast bacillus.

6. Animal experiments undertaken for the purpose of differentiating the acid-fast organisms and to fix their etiological status are not regarded by us as conclusive.

7. Serological tests, especially those performed with highly immune sera, have proved of some value and tend to show that Clegg's bacillus of leprosy is not related to the ordinary acid-fast chromogenic saprophytes, and that the non-chromogenic lepra culture of Duval is different both from Clegg's organism and from all other acid-fast bacilli.

8. The rôle played by the chromogenic bacillus of Clegg in the production of leprosy is as yet an unsettled question, although we are at present inclined to ascribe to it a minor if not a negligible part.

9. The non-chromogenic strain, while behaving according to most of our notions regarding a pathogenic organism, has likewise not up to the present been conclusively proved the cause of leprosy, although we are impressed with the probability of such a rôle being eventually attributed to it and consider that it deserves more serious attention than any organism so far cultivated from the human leprous lesion.